

REMARKS

Claims 1, and 6-8 are pending. New claim 25 is added by this amendment.

Restriction of Previously-submitted Claims 9-24

The Examiner contends that previously-submitted claims 9-34 are directed to an invention that is distinct or independent from the originally claimed invention since these claims are drawn to distinct species of target, proteins, or modifiers which were not elected. The Examiner acknowledges that claim 1 is a genus claim and claims 9-24 are species of the genus.

In response to this statement, Applicants these claims have been withdrawn. However, should generic claim 1 be found allowable following this submission, it is requested that the Examiner require an election of species pursuant to MPEP section 809.02(b), followed by a full action on the merits for the elected species.

Rejection under 35 U.S.C. §102(b)

Claims 1, 6 and 7 have been consistently rejected by the Examiner as anticipated by Li et al. *PNAS USA* 1997; 94: 73-78 ("Li"). In an Advisory Action dated February 11, 2004, the Examiner declined to enter the previous Amendment (after Final) submitted on January 14, 2004, in which several amendments to claim 1 were proposed. Accordingly, by this amendment, claim 1 has been amended in view of the Examiner's comments in the Advisory Action. This is discussed further below following a discussion of Li.

As set forth repeatedly in previous responses, Li discloses a computer-based strategy for identifying compounds that bind to a functionally critical site (an "epitope") on the surface of CD4. This binding site is defined by Li as the site where the CD4 receptor interacts (*i.e.*, binds) with the MHC II antigen on the antigen-presenting cell (see page 74, column 1). Li defines the functional epitope by identifying CD4 mutants that exhibit differential binding to monoclonal antibodies or MHC molecules.

In the Methods section on page 74, Li refers to an article published in 1990 by Ryu et al. which describes the high-resolution X-ray structure of the human CD4 D1 domain. According to the Ryu article, “residues implicated in HIV recognition by analysis of mutants and antibody binding are salient features in domain D1” (emphasis supplied). Specifically, Li determined that the large surface area of CD4 D1, and specifically loops designated FG and C’C”, were involved in a direct interaction with MHC II. Accordingly, the recognition-site in domain D1 of CD4 is a functionally critical site, *i.e.*, a site involved in direct interaction with a ligand (MHC II). The use of the term epitope by Li is understood in the literature to mean a site determined by *either* antibody binding *or* by T cells after antigen presentation, it is not a site that is defined as a cleft that lies distal to an active site.

Thus, the compounds identified by Li disrupt the CD4/MHC interaction by a competitive mechanism, *i.e.*, the compounds bind in the functionally critical site and prevent binding between the natural ligand and the receptor. This is supported by Li’s statement on page 74 that his proposed binding-pocket was consistent with the available mutational data by Fleury et al. that the CDR-MHC interaction occurs at an **epitope** comprising amino acid residues 19, 89 and 165 on exposed loops of domain D1. This is further supported by Li’s statement that the four disclosed inhibitors inhibit the CD4-MHC II interaction in a concentration-dependent manner (see page 75-76, bridging sentence), which is characteristic of competitive inhibition and not allosteric inhibition. Moreover, in the discussion on page 77 (column 2), Li states that:

...we have proposed that the CC’ loop, together with the FG (CDR3) loop, form a critical binding pocket on the lateral surface of CD4. It is interesting to note that similar surface pockets on other Ig-related proteins are also involved in molecular interactions and biological functions ...a similar pocket consisting of the FG and CC’ loops is commonly observed to be involved in dimerization of Ig superfamily molecules...this pocket also mediates heterophilic interactions ...The results of our study regarding the role of the CD4 surface pocket in mediating stable MHC class II interaction and T-cell activation are consistent with this notion (*emphasis not original*).

site of interaction between a peptide mimetic and CD4, which cavity could accommodate a 1.4 angstrom water probe. The present invention does not require use of antibodies or mutagenesis to identify the proximal cavities (i.e., the allosteric sites).

It should therefore be clear from the above that i) the functionally critical site **is not** the same as the proximal allosteric site; and ii) the Li method of identifying the functionally critical site **is not** employed in the method of the present claims. Claim 1 is currently amended in an attempt to clarify to the Examiner that the present invention is directed to identifying a compound that allosterically modulates a protein by binding to the protein at one site (the allosteric site), and altering an interaction at between the protein and a ligand at another site (the functionally critical site). This identification requires first identifying a cavity that is distant from the functionally critical site (i.e. a candidate allosteric cavity), and *then* searching for compound which bind in the candidate allosteric site and disrupts binding of the ligand to the protein at the functionally critical site. Support for the inclusion of the term “allosteric modulator” can be found on page 4, line 17, to page 5, line 2.

Since a reference cannot **anticipate** if it does not disclose each and every claim limitation, and since Li does not mention allosteric modification (but is instead concerned solely with competitive inhibition at the functionally critical site), Li cannot anticipate the present claims. Li also does not anticipate the present claims because Li’s method of identifying the functionally critical site relies on epitope mapping and mutagenesis. Li does not disclose a size-based method of employing a 1.4 angstrom water probe to identify a cavity, much less an allosteric cavity. Accordingly, withdrawal of this rejection is respectfully requested.

Rejections under 35 U.S.C. §112-Enablement

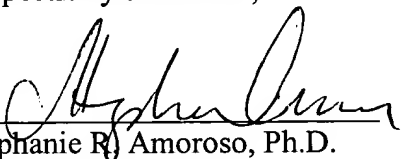
Claim 1 stands rejected for lack of enablement. The Examiner contends that in order to practice the claimed screening method depends one must first obtain a crystal structure of the target protein. The Examiner did not find Applicant’s previous arguments regarding enablement to be persuasive since the Examiner alleges that crystallization as of the filing date was unpredictable.

that once an allosteric cavity and a candidate allosteric modulator (determined to bind in the cavity) are identified, determining whether the candidate allosteric modulator inhibited or enhanced activity of the target protein would require the same amount of routine experimentation. For example, a candidate compound used in the assays described in the specification would reveal both inhibitory effects and enhancer effects. Allosteric enhancers are known in the art (see attached abstract of Lanzereno et al.). Accordingly, withdrawal of this rejection is respectfully requested.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

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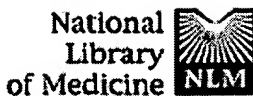


improper to come to a conclusion of non-enablement based on analysis of only *one* of the *Wands* factors.

The Examiner also rejects the claims as non-enabled for allegedly exceeding the scope of the disclosure. The Examiner contends that only the three proteins exemplified, TNF- α , CD4, and β -lactamase, are enabled by the specification.

Again, Applicants have previously set forth the detailed arguments against this rejection, which clearly have been misunderstood by the Examiner. While Applicants did provide evidence for the predictability of crystallization as of the filing date, this was only ancillary to the Applicants' main point that prediction of the tertiary structure of proteins using several well-known techniques was enabled as of the filing date. This evidence was not support for the premise that crystallization is necessary to practice the presently claimed method. As indicated in the previous response, the state of the art as of the filing date was quite advanced with respect to tertiary structure prediction using **any** of the enumerated methods above (as evidenced by the previously-submitted documents and the teachings of the specification). In addition, the likelihood that proteins discovered subsequent to the filing date would be homologous with at least one protein whose tertiary structure was known, is a given considering the thousands of proteins whose structures were known as of the filing date (see previous response).

Further, as pointed out previously, the Examiner is incorrect in citing the Examples as the sole enablement for the claimed invention. There is no statutory requirement for the working examples, and although the examples may represent the "best mode" of the invention, their presence does not bar claiming other, unexemplified embodiments of an invention as long as the embodiments are sufficiently described and enabled. This is the case in the present application. The examples (of 3 different classes of proteins) and the balance of the specification enable the full breadth of the present claims.

Lastly, the Examiner rejected claim 8 as not enabled for reciting that the allosteric modifier could be an enhancer and not an inhibitor. To address this rejection, Applicants submit



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☐ 1: J Mol Neurosci. 2003;20(3):363-7. Related Articles, Links

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Progress toward a high-affinity allosteric enhancer at muscarinic M1 receptors.

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Loss of forebrain acetylcholine is an early neurochemical lesion in Alzheimer's disease (AD). As muscarinic acetylcholine receptors are involved in memory and cognition, a muscarinic agonist could therefore provide a "replacement therapy" in this disease. However, muscarinic receptors occur throughout the CNS and the periphery. A selective locus of action of a muscarinic agonist is therefore crucial in order to avoid intolerable side effects. The five subtypes of muscarinic receptors, M1-M5, have distinct regional distributions with M2 and M3 receptors mediating most of the peripheral effects. M1 receptors are the major receptor subtype in the cortex and hippocampus—the two brain regions most associated with memory and cognition. This localization has led to a, so far unsuccessful, search for a truly M1-selective muscarinic agonist. However, acetylcholinesterase inhibitors, such as donepezil (Aricept), which potentiate cholinergic neurotransmission, do have a therapeutic role in the management of AD and so the M1 receptor remains a viable therapeutic target. Our approach is to develop muscarinic allosteric enhancers—compounds that bind to the receptor at an "allosteric" site, which is distinct from the "primary" site to which ACh binds, and which enhance ACh affinity (or efficacy). Having discovered that a commercially available compound, WIN 62577, is an allosteric enhancer with micromolar potency at M3 receptors, we report here some results of a chemical synthesis project to develop this hit. Modification of WIN 62577 has led to compounds with over 1000-fold increased affinity but, so far, none of these extremely potent compounds are allosteric enhancers.

MeSH Terms:

- Acetylcholine/agonists
- Acetylcholine/deficiency
- Allosteric Site/drug effects*
- Allosteric Site/physiology